Running title: UV light for reduction of *Salmonella* on eggs

Comparison of UV-C and pulsed UV light treatments for reduction of *Salmonella*, *Listeria monocytogenes* and enterohemorrhagic *Escherichia coli* on eggs

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ABSTRACT

Ten per cent of all strong-evidence food-borne outbreaks in the EU are caused by *Salmonella* related to eggs and egg products. UV light may be used to decontaminate egg surfaces and reduce the risk of human salmonellosis infections. The efficiency of continuous UV-C (254 nm) and pulsed UV light for reducing the viability of *Salmonella* enteritidis, *Listeria monocytogenes* and enterohemorrhagic *Escherichia coli* on eggs was thoroughly compared. Bacterial cells were exposed to UV-C light at fluences from 0.05 to 3.0 J/cm^2 (10 mW/cm^2, from 5 to 300 s) and pulsed UV light at fluences from 1.25 to 18.0 J/cm^2, resulting in reductions ranging from 1.6 to 3.8 log depending on conditions used. Using UV-C light it was possible to achieve higher reductions at lower fluences compared with pulsed UV light. When *Salmonella* were stacked on a small area or shielded in feces, the pulsed UV light seemed to have a higher penetration capacity and gave higher bacterial reductions. Microscopy imaging and attempts to contaminate the interior of the eggs with *Salmonella* through the eggshell, demonstrated a maintained integrity of the eggshell after UV light treatments. Only minor sensory changes were reported by panelists when the highest UV doses were used. UV-C and pulsed UV light treatments appear to be useful decontamination technologies which can be implemented in continuous processing.

INTRODUCTION

Although producers strive to keep foods safe, food poisoning outbreaks still occur. In 2015, there were 95,000 registered cases of *Salmonella* in the EU, with a prevalence of 21 cases/100,000 individuals. *Salmonella* in eggs was associated with the highest number of reported food-outbreaks and accounted for 10% of all strong-evidence outbreaks in the EU *(19)*. Foodborne outbreaks are registered in the EU's RASFF (Rapid Alert System for Food and Feed) and the EWRS (Early Warning and Response System) databases for enhanced awareness during the outbreaks. The prevalence of *Salmonella* on eggshells is around 0.03% on single samples and 0.5% on batch samples *(18).* Contaminated eggshells are more important than internal *Salmonella,* for which the prevalence is only around 0.005%. Not all eggs from *Salmonella* infected flocks are necessarily contaminated (often less than 3% of the eggs). Due to the high number of food poison cases, safety of eggs are prioritized in a coordinated approach from the EU.

Eggs can become contaminated with bacteria from the hen's intestinal tract, feces, infested nests, or from the surrounding environment including air and conveyor belts during production *(12, 51).* The bacteria may penetrate the shell, contaminate internal contents of the egg and cause damage or death to the embryo. The number of bacteria on eggs vary with 4-5 log CFU/egg depending on the hygienic conditions *(31)*. *Salmonella* infectious dose is 10^2-10^3 bacteria, but can be as low as 15-20 bacteria *(7).* Implementation of control strategies for egg production *(4),* has successfully led to reduced incidences of food poisoning *(5).*

Sanitization of eggs has traditionally been done either by washing in water *(30)* or by immersing in disinfectant solutions. Washing of grade A table eggs is generally not allowed in the EU *(3).* The major disadvantage of egg washing is the potential damage to the physical

barriers, such as the cuticle, which may favor trans-shell contamination with bacteria *(17)*. Sanitation of hatching eggs is required to reduce problems with decreased hatchability, poor chick quality and growth *(48)* and cross contamination during hatching *(11)*. Commonly used disinfectants for hatching eggs involve formaldehyde fumigation or spraying with solutions containing chlorine-based or quaternary ammonium compounds *(20)*. A number of other decontamination strategies for eggs have been examined including use of N-halamine compounds, electrolyzed oxidative water, ozone, ionized air, sodium hydroxide and hydrogen peroxide *(14, 51, 55)*. Eggs will be downgraded in the EU if any forms of disinfections are used *(55)*.

In recent years, the use of UV light as a surface decontamination method has been met with increasing interest *(51)*. Regulations in conjunction with using conventional continuous UV-C light and pulsed UV light in the USA are given by the U.S. Food and Drug Administration (FDA) *(22)*. UV-C light can be employed in the EU, however, in Germany the use is limited to water, fruit and vegetable products and stored hard cheeses *(2)*. UV-C light, primarily at 254 nm, provides effective inactivation of microorganisms by damaging nucleic acids creating nucleotide dimers, and thus leaving the microorganisms unable to perform vital cellular functions.

When a mixture of *Salmonella* Typhimurium and four other serovars were subjected to UV-C light at 9 mW/cm^2 at a fluence of 0.14 J/cm^2, 3-4 log reduction was observed *(23)*. Coufal et al. obtained a 4 log reduction of a strain of *Salmonella* Typhimurium on hatching eggs after a 3.4 J/cm^2 exposure *(13)*. In contrast, only 1 log reduction of *Salmonella* was obtained after a 4 J/cm^2 UV-C treatment when eggs were stored for two days prior to UV-C treatment *(49)*. When a strain of *Escherichia coli* and a *Listeria monocytogenes* strain were treated with UV-C light at 0.18 J/cm^2, 4 log reduction was obtained *(15)*. Total aerobic plate counts were reduced by 1-3 log when subjected to UV-C at approx. 0.4 J/cm^2 *(12)*. For a

review see Turtoi and Borda *(51)*. Other papers also describe the use of UV-C light on eggs in combination with other methods such ozone treatment *(46)* and H_2O_2 *(1)*.

High intensity pulsed UV light has been approved by the FDA up to 12 J/cm^2 as a means for controlling surface microorganisms on food products. The UV energy spectrum of pulsed UV light is caused by bremsstrahlung and covers the whole spectrum from UV (200 nm) into the infrared region (1100 nm). In addition to creating nucleotide dimers, pulsed UV light has been suggested to cause cell death by induction of cell membrane damage *(50)* and rupture of the bacteria by overheating caused by absorption of all UV light from the flash lamp *(52)*. Also, disturbances caused by high-energy pulses have been suggested to contribute to cell damage *(34)*.

Varying result have been obtained when using pulsed UV light for decontamination of *Salmonella* on eggs. Dunn et al. obtained >7.9 log reduction when a strain of *Salmonella* Enteritidis was exposed to a fluence of 4 J/cm^2 *(16)*. In contrast, Hierro et al. observed only 1 log reduction using the same fluence, and 2 J/cm^2 giving only 0.14 log reduction *(28)*. When *Salmonella* was subjected to 1.2 - 35.3 J/cm^2, reductions ranged from 2 - 7.7 log, the high value reached after a 30 s treatment using 90 pulses *(32)*. A sigmoidal model for the inactivation of *Salmonella* on eggs has been developed *(33)*. Factors affecting the efficacy of pulsed UV light for pathogens have been investigated by treating bacteria on petri dishes under different conditions *(21)*.

The effectiveness of UV-C and pulsed UV light for decontamination depends on the length of time a microorganism is exposed, the intensity and wavelength of the illuminaton, the microorganism's ability to withstand the UV exposure, properties of the food surface, the penetration of the UV light and the presence of particles shielding the microorganisms. Varying results have previously been reported. In the present study, the efficiency of UV-C and pulsed UV light against bacteria on eggs under different conditions using the same sets of

cells treated identically before various UV light treatments was compared. To our knowledge, a thorough comparison of the two methods for reducing bacteria on eggs has previously not been published. By using several strains of each species of *Salmonella* Enteritidis, *Listeria monocytogenes* and enterohemorrhagic *Escherichia coli*, the aim was to obtain results representative for the species. The importance of presence of dry matter, presence of feces and concentration of cells on efficiency of UV light treatment of eggs, which have previously not been studied, was also investigated. Moreover, the effects of different treatments on eggshell integrity was investigated and the sensory changes of the eggshell and egg content as a result of the UV exposures were evaluated. All these elements are important for practical implementation of UV technology in the industry.

MATERIALS AND METHODS

Bacterial strains, media and growth conditions. Three strains of *Salmonella* Enteritidis, four strains of enterohemorrhagic *E. coli* (EHEC) and four strains of *L. monocytogenes* were used (Table 1). The strains were maintained at -80°C in growth medium supplemented with 20% glycerol (v/v). Rifampicin resistant (Rif^R) derivatives were prepared by growing strains in liquid media containing 200 µg/ml rifampicin (Rif, Sigma-Aldrich, St. Louis, USA) as described by Heir et al. *(27)*. Growth of the wild types and the Rif^R strains were identical in their respective media and growth conditions when tested using a Bioscreen C instrument (Growth Curves Oy, Helsinki, Finland). The strains were cultured separately in tryptic soy broth (TSB, Oxoid, Hampshire, UK) with 200 µg/ml Rif (16 h incubation, 37°C) to $6x10^8$ cfu/ml, and thereafter the different strains of the same species were mixed in equal amounts before the decontamination experiments.

UV treatment of eggs inoculated with bacterial cells. Fresh eggs were purchased from local retail stores. The surface of eggs were contaminated with approx. 10^7 CFU bacteria in 15 µl broth by spreading with the pipette tip on approx. 8 cm^2 unless otherwise stated. In some experiments, eggs were contaminated with 10^7 CFU/egg of bacteria mixed with chicken feces, where 10, 20 and 40 µl of contaminated feces were spread on a 4 cm^2 area, giving average thicknesses of about 0.025, 0.05 and 0.1 mm, respectively. Contaminated eggs were left at room temperature to dry for approx. 1 hr prior to UV light treatment. In the continuous UV-C light experiments, samples were treated in a custom made aluminum chamber (1.0x0.5x0.6 m^3) equipped with two UV-C lamps (UV-C Kompaktleuchte, 2x95 W, Bäro GmbH, Leichlingen, Germany) in the ceiling. The UV-C light was emitted essentially at

253.7 nm, and the intensity was measured using a UVX Radiometer (Ultra-Violet Products Ltd., Cambridge, UK) with a UV-C sensor (model UVX-25, Ultra-Violet Products Ltd., Cambridge, UK). Samples were exposed at a power intensity of 10 mW/cm^2, which is close to a maximum when using commercial lamps, and exposure times were 5, 10, 30, 60 or 300 s, giving fluences of 0.05, 0.1, 0.3, 0.6, 3.0 J/cm^2, respectively. For pulsed UV light treatments, the instrument XeMaticA-SA1L (SteriBeam Systems GmbH, Kehl-Kork am Rhein, Germany) was employed. The instrument was equipped with a xenon flash lamp (19 cm), which was water cooled, with an aluminum reflector (with opening 10 cm x 20 cm), and emitted light of 200-1100 nm with up to 45% of the energy being in the UV-light region with maximal emission at 260 nm for high energy pulses (SteriBeam Systems GmbH, Kehl-Kork am Rhein, Germany). Eggs were illuminated at 6.5 cm distance barely beneath the opening of the reflector. At this distance, the fluence could be calculated according to the manufacturer's specifications as the total discharge energy of the lamp divided by the opening area of the reflector The fluence of each pulse was adjusted to 1.25 J/cm^2 (low) or 3.6 J/cm^2 (high) by adjusting the discharge voltage. The samples were exposed with single pulses either once to the low pulse, or one, three or five times to the high pulse (3.6, 10.8 or 18.0 J/cm^2), respectively. After illumination, the treated areas were swabbed to collect surviving bacteria. Temperatures were measured using a Raynger MX infrared thermometer (Raytek Corporation, Santa Cruz, USA). The experiments were performed in a Biosafety level 3 pilot plant.

Analysis of cells. After UV light treatments, the eggs were swabbed, and the collected surviving bacteria were plated onto tryptic soy agar (TSA, Oxoid, Hampshire, UK) with 200 µg/ml Rif using an automated plate spreader (Whitley Automatic Spiral Plater, Don Whitley Scientific Ltd., West Yorkshire, UK) and incubated over night at 37°C. The number of

colonies were determined using an automatic plate reader. Since Rif^R strains were used, the background flora on the eggs were negligible.

Effect of UV-C light intensity. The effect of the UV-C light intensity on reduction of *Salmonella* was determined by exposing contaminated eggs to pairwise similar fluences. Treatments were 10 mW/cm^2 for 5 s and 2 mW/cm^2 for 25 s, (fluence of 0.05 J/cm^2 in both cases), and 10 mW/cm^2 for 30 s and 2 mW/cm^2 for 150 s (fluence 0.3 J/cm^2 in both cases).

Photoreactivation. Eggs were contaminated with 10^6 CFU *Salmonella* and exposed to UV-C light at a fluence of 0.05 J/cm^2 (10 mW/cm^2 for 5 s) or pulse UV light at 1.25 J/cm^2. For detection of photoreactivation, the treated eggs were thereafter either subjected to low or a high UV-A dose as follows: eggs were subjected to low intensity UV-A light for 3 h at room temperature in a dark room using a TW6W Black light Blue lamp (emission spectrum in the 300-400 nm range with maximum at 365 nm, Heraeus Noblelight, Cambridge, UK) at approx. 0.01 mW/cm^2, or high UV-A dose by using a Black Light Euroline UV-A lamp at approx. 0.9 mW/cm^2 (35 W, Steinigke Showtechnic GmbH, Waldbüttelbrunn, Germany) at a distance of 40 cm. Appropriate controls (exposed to the same UV-C or pulsed UV light doses) were stored in the dark for 3 hours. The eggs were thereafter swabbed and cells were plated on TSA with 200 µg/ml Rif and incubated at 37°C over night. For testing whether photoreativation could be detected under optimal conditions, eggs were exposed to UV-C and pulsed UV light as described above, before being immediately swabbed to collect bacteria. The collected bacteria were plated on TSA with 200 µg/ml Rif and subjected to the UV-A treatments as described above while on the petri dishes (with the lids removed) rather than on dry eggs, before incubation at 37°C over night.

Testing for *Salmonella* eggshell transfer after UV light treatment. Eggs were illuminated with 3.0 J/cm^2 (10 mW/cm^2 for 5 min) UV-C light or 10.8 J/cm^2 pulsed UV light. Testing for bacterial transfer, the illuminated area was thereafter contaminated with 10^7 CFU *Salmonella* and incubated for 24 h at room temperature in a closed box. Non-illuminated controls contaminated on the outside were kept in the same box and as positive controls, two eggs were contaminated in the egg white. After incubation, the pointy end of the egg was flame sterilized for 10 s, a hole was made using a sterile scalpel and the egg content was transferred to a stomacher bag, essentially as described by Himathongkham et al. *(29).* The content was homogenized for 2 min and 25 g was removed for qualitative detection of *Salmonella* according to NMKL ISO 6579:2002 *(40).*

Scanning electron microscopy. Eggs were exposed to UV-C light or pulsed UV light. Eggshell samples were thereafter coated with gold/palladium by using a Polaron Sputter Coater (SC 7640, Leica Microsystems, Wetzlar, Germany). The coated samples were examined and photographed with a Zeiss EVO-50-EP scanning electron microscope (Zeiss Microscopy GmbH, Jena, Germany) at an accelerating voltage of 20 kV in the secondary emission mode.

Sensory evaluation. Ten well trained sensory assessors were selected and educated according to recommendations in ISO 8586:2012 (*General guidelines for the selection, training and monitoring of selected assessors, and expert sensory assessors*), and ISO 13299:2003 (*General Guidance for establishing a sensory profile*). Panelists were given treated intact eggs for examination, eggs were then broken and the interior was examined. The

samples were: untreated control, eggs treated with UV-C light at 0.1 J/cm^2 (10 mW/cm^2, 10 s), eggs treated with UV-C light at 0.6 J/cm^2 (10 mW/cm^2, 60 s), eggs exposed to pulsed UV light at low intensity (1.25 J/cm^2) and eggs exposed to pulsed UV light 3 times at high intensity (10.8 J/cm^2). The samples were served at room temperature on white dishes identified by random three-digit numbers. Each panelist recorded their results at individual speed on a 15 cm non-structured continuous scale with the left side of the scale corresponding to the lowest intensity, and the right side scale corresponding to the highest intensity. The computer transformed the responses into numbers between 1 = low intensity, and 9 = high intensity. Samples were served in two replicates and in randomized order following a balanced block experimental design. Sixteen sensory attributes were evaluated in the descriptive sensory analysis of the raw eggs: Odour of the eggshell (chalk, sunburn, burnt, metallic, sulphur, hen house, cloying), odour of broken egg (sourness, sunburnt, burnt, metallic, sulphur, sweetish, cloying), and appearance of egg content (transparency of egg white and clarity of egg yolk).

Statistical analysis. Three parallels of UV light treated samples and three or six untreated controls were used in each experiment. The experiments were repeated three times on different days. Analysis of variance (ANOVA) was used to determine statistically significant effects on the bacterial reduction by the treatments. All analyses were performed in R *(45)*. In order to identify the sensory attributes that discriminated between samples, ANOVA using a two-way model with interactions and with the assessor and interaction effects considered random, was performed on the descriptive sensory data from the trained panel. The statistical software used in sensory analysis was EyeOpenR® (Logic8 BV, Utrecht, the Netherlands).

RESULTS

Reduction of *Salmonella* on clean eggs. *S.* Enteritidis strains in growth medium were mixed and applied to the surface of clean eggs. After drying, the eggs were subjected to various continuous UV-C and pulsed UV light treatments, resulting in bacterial reductions between 2.3 log and 3.8 log, depending on the UV dose (Fig. 1 and Supplemental material Table S1). Some increase in reduction could be obtained by increasing the UV dose, but this strategy appeared limited as was also indicated from Weibull models constructed from the reduction data (Suppl. Material, Table S2, Fig. S1). For the UV-C light treatments, increasing the dose 60 fold only gave a 1.1 log increase in reduction. By comparing the UV-C light results using ANOVA, the shortest treatment at 0.05 J/cm^2 was considered statistically different from the treatment of the longest duration and 3.0 J/cm^2. Likewise, some increase in reduction could be observed from low intensity of 1.25 J/cm^2 to high intensity of 3.6 J/cm^2 for the pulsed UV light exposures, however, statistically, no additional increase was achieved by further increasing the fluence to 10.8 and 18.0 J/cm^2. In control experiments where *Salmonella* was spread on agar plates and subjected to the same treatments, 5-6 log reduction was obtained even with the mildest UV treatments.

Coinciding results were obtained with cells washed twice in distilled water and thereafter spread on a similar area. The reduction was 2.5 and 2.1 log after UV-C light treatment at 0.05 J/cm^2 and pulsed UV light treatment at 1.25 J/cm^2, respectively. Increasing the UV doses further enhanced the reduction to around 3 log for both UV methods. When comparing the UV reduction results from washed and unwashed cells by ANOVA, no significant differences were found. However, when the same amount of washed bacteria were applied to a small area, significantly lower reduction was achieved after UV-C light treatment, with only 0.8 log reduction after a 0.6 J/cm^2 treatment, increasing to 1.7 log after treatment at

3.0 J/cm^2. For pulsed UV light, the reduction increased with increasing UV fluence from 0.7 log at 1.25 J/cm^2 to 3.5 log at 18 J/cm^2. The temperature increase of the eggs during UV treatments was negligible, transiently at most a few °C on the exposed surface.

Reduction of *Salmonella* on dirty eggs. The *Salmonella* strains were mixed with chicken feces, which was applied to eggs and subjected to UV-C and pulsed UV light treatments (Fig. 2). Reductions of bacteria in feces were lower when comparing with the corresponding treatments of bacteria in growth medium or water. Between 0.8 and 2.2 log reduction was observed, depending on the UV dose and the thickness of the feces layer. For the UV-C light, 0.9 log reduction was obtained after the 0.05 J/cm^2 treatment in the thinnest feces layer, increasing to 1.9 log after the 0.6 J/cm^2 treatment. For the thicker layers, reductions remained at 0.8-1.0 log regardless of treatment. Pulsed UV light exposure at low fluence (1.25 J/cm^2) gave reductions of 0.4-1.0 log depending on the feces thicknesses. Clearly, an increased reduction for all feces layers was observed when increasing the fluence, with reductions ranging from 1.3 to 2.2 log, indicating a higher penetration of the pulsed UV light.

Reductions of *L. monocytogenes* and EHEC. Strains of *L. monocytogenes* and EHEC in growth medium were applied to the surface of clean eggshells, as described above for *Salmonella*. After drying, the eggs were subjected to UV-C light and pulsed UV light treatments (Fig. 3). Reduction of *L. monocytogenes* was between 1.8 and 3.7 log, depending on the UV dose. By comparing the UV-C light results using ANOVA within *L. monocytogenes*, only the 0.05 J/cm^2 and the 3.0 J/cm^2 treatments were considered statistically different, indicating that increasing the UV dose will give minor increase in reductions, as

was also apparent from the Weibull models constructed from the reduction data (Fig. S2). Similarly, for the pulsed UV light treatment, only the 1.25 J/cm^2 treatment was considered statistically different from the other pulsed UV light treatments, and increasing the fluence beyond 3.6 J/cm^2 gave little increase in reduction. Similar results were obtained with the EHEC strains tested. Reductions ranged from 1.6 to 3.7 log depending on the treatment. For the UV-C light, testing for EHEC separately, only the treatment at 3.0 J/cm^2 was statistically different from the other treatments. For pulsed UV light, the 1.25 J/cm^2 treatment gave a lower reduction than the higher fluence treatments.

When comparing similar treatments for *Salmonella*, *L. monocytogenes* and EHEC using ANOVA, the different bacterial species showed very similar sensitivities against both continuous UV-C light and pulsed UV light. In control experiments where the same *L. monocytogenes* and EHEC strains were spread on agar plates and subjected to the same treatments, generally between 5 and 6 log reductions were obtained even after the mildest UV treatments, and similar to the results for *Salmonella*.

Effect of UV-C light intensity. Treatments at 10 mW/cm^2 for 5 s and at 2 mW/cm^2 for 25 s, giving a fluence of 0.05 J/cm^2 in both cases, resulted in 2.7 and 3.2 log reduction, respectively. No statistical difference was detected by ANOVA between the two treatments. For treatments at 10 mW/cm^2 for 30 s and 2 mW/cm^2 150 s (both 0.3 J/cm^2), which gave reductions of 3.2 an 4.1 log, respectively, a statistical difference was observed with higher reduction at the lower intensity and longer exposure time.

Testing for photoreactivation. Photoreactivation experiments were carried out to determine if cells exposed to UV-C or pulsed UV light were dead or could be rescued by

induction of UV-A light inducible repair systems. *Salmonella* on eggs which had been treated with UV-C light at fluence 0.05 J/cm^2 (10 mW/cm^2 for 5 s) or pulsed UV light at 1.25 J/cm^2, showed no photoreactivation when thereafter being exposed to two different fluences of UV-A light for 3 h (results not shown). For *Salmonella* on eggs treated similarly with UV-C or pulsed UV light, but transferred to petri dishes to provide optimal opportunities for photoreactivation, before the dishes were exposed to the same two intensities of UV-A light, also did not lead to increased survival by photoreactivation, thus the cells seemed to be killed and not damaged when exposed to UV-C and pulsed UV light at the fluences tested.

Eggshell integrity. The integrity of the eggshells after UV light treatments were examined by exposing eggs to UV-C light at 3.0 J/cm^2 (10 mW/cm^2, 5 min) or pulsed UV light at 10.8 J/cm^2. The eggs were subsequently contaminated with *Salmonella* and left for 24 h before the egg content was examined for *Salmonella* passing through the shell (Table 2). No *Salmonella* were detected inside the eggs, indicating that the UV treatments do not change the barrier properties of the eggshell. To further investigate this, eggs exposed to UV-C light at 0.6 J/cm^2 (10 mW/cm^2, 60 s) and pulsed UV light at 10.8 J/cm^2 were examined by scanning electron microscopy (Fig. 4). No changes were visible in the proteinaceous cuticle after the UV treatments compared to the untreated controls.

Sensory evaluation. Ten trained assessors evaluated eggs exposed to UV-C light at 0.01 and 0.6 J/cm^2 (10 mW/cm^2, 10 s and 60 s, respectively), and pulsed UV light at 1.25 and 10.8 J/cm^2. Treated intact eggs at room temperature were examined for odour (Fig. 5). The eggs were then broken and the egg contents were examined for odour and transparency. Generally, small differences were registered for intact eggs treated with UV light. Most

notably, treatment with the highest fluence of pulsed UV light gave a higher intensity of sunburnt odour, sulphur odour and henhouse odour compared to the untreated controls. Sunburnt odour is associated with that of sunburnt human skin. Some increase in this attribute was also found for the egg content after exposure to the highest doses of both UV-C light and pulsed UV light. However, a sensory intensity value of two is considered very low.

DISCUSSION

To avoid possible changes in sensory perception, it is desirable to maximize the reduction of bacteria without treating the eggs more than necessary. The fluence treatment levels for UV-C light were selected from low levels suitable for practical use, where limited kill of bacteria were observed, up to fluences comparable to those of the pulsed UV light. Pulsed UV light was tested at fluences from 1.25 J/cm^2 up to and above the limit value of 12 J/cm^2 determined by FDA. The fluences of the two methods are not directly comparable since the different wavelengths in the UV spectrum have different germicidal effectiveness *(9)*. For bacteria spread over a large area on the egg, the germicidal effect of UV-C light with a fluence of 0.05 J/cm^2 was comparable to a fluence of 1.25 J/cm^2 for the pulsed UV light (Fig. 1). The higher germicidal effect at lower fluence for the UV-C light is likely explained by most of the energy being emitted at 254 nm, where relative germicidal effect is close to the maximum *(9)*. The germicidal effect also seemed to be influenced by intensity-time combinations, as seen in the difference in reduction at pairwise similar fluences. Limited dose-response effects in the ranges tested is likely caused by shading effects of the irregular surface structure of the eggs *(53)*. The efficacy of using UV light for decontamination of foods is often lower than when tested on clean surfaces *(26)*. UV light does not penetrate well through organic matter, such as protein and other organic matrices, and the penetration into

opaque matter seems to depend on the type of matter. Medium components did not shield cells from UV exposure, agreeing well with result obtained when bacteria dissolved in fetal calf serum were plated on petri dishes, where no differences in levels of inactivation were observed *(21)*. Pulsed UV light has been reported to penetrate opaque denatured whey protein isolate down to at least 10 mm *(8)*. However, impaired germicidal effect was evident when bacteria were shielded by fecal matter (Fig. 2), and a higher penetration of pulsed UV light compared with UV-C light was evident. Bacteria stacked on top of each other can also create a shadowing effect, as has been reported for colonies of *L. monocytogenes* growing on petri dishes, where the upper cells of a colony appeared to protect the lower cells against pulsed UV light *(25)*. Such a shadowing effect was clearly observed when bacteria were applied on small spots on the eggs, (Fig. 1C). The highest intensities of pulsed UV light appeared to partly overcome this shadowing effect. This increased reduction may in part also be caused by local overheating of bacteria due to absorption of UV light of many different wavelengths *(34, 52)*.

In accordance with our findings, very high *Salmonella* reductions have been achieved on smooth surfaces. Gomez-Lopez *et al*. reported >6.85 log reduction on petri dishes after pulsed UV light treatment *(25)* and Pasckeviciute *et al*. observed 6.5-7 log reduction *(42)*. Our results on eggs were in good agreement with several previous reports. When eggs were treated with UV-C light at fluences 0.15, 0.45 and 0.75 J/cm^2 (2.5 mW/cm^2 for 1, 3 and 5 min, respectively), reductions from 3.0 to 4.3 log were obtained *(46)*. These resulting reductions were not statistically different from each other, which reflect the often relatively large variation probably due to differences of the egg surface. Gao *et al.* obtained 2.5-4 log reduction of *Salmonella* on eggs after a treatment at 0.09 J/cm^2 (9 mW/cm^2, 10 s) depending on contamination titer *(23)*. When eggs were treated at fluences 1.5 and 3.1 J/cm^2 (1.72 mW/cm^2 for 15 and 30 min, respectively), 2 log reduction was observed regardless of

treatment time *(35)*. Pertaining to pulsed UV light, large variations in germicidal effect have previously been presented. Dunn reported >7.97 log reduction of *Salmonella* on unprocessed eggs, employing a fluence of 4 J/cm^2 *(16)*. Varying degrees of reduction of a *Salmonella* Enteritidis strain were obtained, from 1.3 log after 1 s treatment to >5.3 log reduction after 20 s treatment at fluence 23.6 J/cm^2 *(32)*. A strain of *Salmonella* Typhimurium was shown to be reduced up to 5 log at fluence 2.1 J/cm^2 *(36)*.

Around 30% of the eggs produced in the EU are processed. *L. monocytogenes* and *E. coli* are considered microbiological hazards in the egg processing industry *(6)*. Different bacteria have been shown to vary in their sensitivity to pulsed UV light, with *L. monocytogenes* showing higher resistance *(21, 25, 37)*. In the present investigation, however, no differences in sensitivity to neither UV-C nor pulsed UV light on eggs between the three mixes of *Salmonella, L. monocytogenes* and EHEC strains could be seen.

Cells commonly have UV-A inducible repair systems to correct damages in their DNA. Photoreactivation studies could indicate whether bacteria are killed or only damaged after UV light treatment. Hierro *et al*. reported photoreactivation of a *Salmonella* Enteritidis strain on noble agar petri dishes after a pulsed UV light treatment of up to 0.525 J/cm^2 *(28)*. A 10 fold lower fluence for the UV-C treatment was chosen in the present work, and . no photoreactivation was detected under the conditions tested, indicating that *Salmonella* were killed even with the mildest treatments. Photoreactivation of *E. coli* on petri dishes after UV-C treatment has been described previously *(41, 44, 54)*. For *L. monocytogenes* on agar plates, subjected to pulsed UV light and subsequently illuminated with fluorescent light, photoreaction has also been observed *(25)*. On the other hand, no photoreactivation was detected when a *Salmonella* Typhimurium strain was exposed to UV-C light on eggs and thereafter treated with fluorescent light for 1 h *(35)*. Likewise, neither a strain of *Salmonella* Typhimurium nor a *L. monocytogenes* strain showed photoreactivation after pulsed UV light

treatment on agar plates followed by UV-A treatment at 365 nm for 1-3 hours *(42)*. It is, however, difficult to directly compare our results and the previously published results since different light sources have been used and often the fluences are not given. The general impression is photoreactivation may occur only after mild UV light treatment.

The cuticle is an important natural physical defense involved in protection of egg contents from invading organisms. Our results from scanning electron microscopy images of the eggshell (Fig. 4) and lack of transfer of *Salmonella* after UV light treatments (Table 2), strongly indicated that the integrity of the eggshell was maintained. Similar results have been reported when a *Salmonella* strain on eggs was treated with 10.5 J/cm^2 pulsed UV light and the egg content was later examined for *Salmonella (36)*. A very low number of *Salmonella* survived the UV light treatment, giving a much reduced probability of transfer compared to the situation in our experiments where a high dose of *Salmonella* (10^7 CFU) was applied to the eggs after pulsed UV light treatment.

Considering the low penetration of UV light into organic matter, limited influence of UV light on the egg content would be expected after illumination of intact eggs. Since changes in odour were small, changes in taste is expected to be insignificant compared to changes occurring when processing eggs (frying, cooking, used in baking etc). A small change in sunburnt odour observed after using the highest UV doses of both UV-C and pulsed UV light implies an upper limit of treatment of eggs in cases where no sensory changes is a requirement. A triangle test performed to detect possible changes in egg white and yolk odour after pulsed UV light treatment of eggs did not reveal any differences. *(36)*. Keklik *et al*. found no changes in albumen height, Haugh unit or the eggshell strength when comparing pulsed UV light treated eggs with control eggs *(32)*. Coloring eggs with a cuticle sensitive dye revealed no visible changes after pulsed UV light treatment *(32)*and changes in rheological

properties have been reported to be insignificant *(36)*. Together these results indicate that sensory changes are small or negligible both after UV-C and pulsed UV light treatments.

In conclusion, both continuous UV-C and pulsed UV light efficiently reduce bacterial levels on the egg surface. The reductions are dependent on the treatment conditions used, and whether the bacteria are directly exposed to the UV light. The UV-C light treatments appear to give higher reductions at lower fluences, while the pulsed UV light shows higher penetration and gives higher reductions for shielded bacteria. The methods can easily be implemented in production and can contribute to reducing the risk of human salmonellosis and other infections.

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SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at:

[URL to be completed by the publisher].

REFERENCES

1. Al-Ajeeli, M. N., T. M. Taylor, C. Z. Alvarado, and C. D. Coufal. 2016. Comparison of eggshell surface sanitization technologies and impacts on consumer acceptability. *Poultry Sci*. 95:1191-7.
2. Anon. 2000. Lebensmittelbestrahlungsverordnung vom 14. Dezember 2000 (BGBl. I S. 1730), die zuletzt durch Artikel 62 der Verordnung vom 31. August 2015 (BGBl. I S. 1474) geändert worden ist. *Jurion* 2125-40-79.
3. Anon. 2003. Commission regulation (EC) No 2295/2003 of 23 December 2003. Introducing detailed rules for implementing Council Regulation (EEC) No 1907/90 on certain marketing standards for eggs. Off. J. Eur. Union. L340:16-34.
4. Anon. 2007. Code of hygienic practice for eggs and egg products CAC/RCP 1976, revision 2007. Codex Alimentarius.
5. Anon. 2009. Special measures to reduce the risk for consumers through *Salmonella* in table eggs - e.g. cooling of table eggs. Scientific opinion of the panel on biological hazards. *EFSA J*. 957:1-29.
6. Anon. 2011. Guide to good manufacturing practice for "liquid, concentrated, frozen and dried egg products" used as food ingredients. European Egg Processors Association, Brugge, Belgium.
7. Anon. 2016, Bad Bug Book: Foodborne pathogenic microorganisms and natural toxins handbook, *Salmonella* spp. http://www.fda.gov/food/foodborneillnesscontaminants/causesofillnessbadbugbook/ucm069966.htm.
8. Bialka, K. L., A. Demirci, P. N. Walker, and V. M. Puri. 2008. Pulsed UV-light penetration of characterization and the inactivation of *Escherichia coli* K12 in solid model systems. *Trans. Asabe*. 51:195-204.

9. Bintsis, T., E. Litopoulou-Tzanetaki, and R. K. Robinson. 2000. Existing and potential applications of ultraviolet light in the food industry - a critical review. *J. Sci. Food Agric*. 80:637-645.

10. Blom, H., E. Nerbrink, R. Dainty, T. Hagtvedt, E. Borch, H. Nissen, and T. Nesbakken. 1997. Addition of 2.5% lactate and 0.25% acetate controls growth of *Listeria monocytogenes* in vacuum-packed, sensory-acceptable servelat sausage and cooked ham stored at 4 degrees C. *Int. J. Food Microbiol*. 38:71-76.

11. Cason, J. A., N. A. Cox, and J. S. Bailey. 1994. Transmission of *Salmonella* Typhimurium during hatching of broiler chicks. *Avian Dis*. 38:583-588.

12. Chavez, C., K. D. Knape, C. D. Coufal, and J. B. Carey. 2002. Reduction of eggshell aerobic plate counts by ultraviolet irradiation. *Poultry Sci*. 81:1132-1135.

13. Coufal, C. D., C. Chavez, K. D. Knape, and J. B. Carey. 2003. Evaluation of a method of ultraviolet light sanitation of broiler hatching eggs. *Poultry Sci*. 82:754-759.

14. Davies, R. H., and M. Breslin. 2003. Investigations into possible alternative decontamination methods for *Salmonella* Enteritidis on the surface of table eggs. *J. Vet. Med. Series B-Infect. Dis.Vet. Publ. Health*. 50:38-41.

15. De Reu, K., K. Grijspeerdt, L. Herman, M. Heyndrickx, M. Uyttendaele, J. Debevere, F. F. Putirulan, and N. M. Bolder. 2006. The effect of a commercial UV disinfection system on the bacterial load of shell eggs. *Lett. Appl. Microbiol*. 42:144-148.

16. Dunn, J. 1996. Pulsed light and pulsed electric field for foods and eggs. *Poultry Sci.* 75:1133-1136.

17. EFSA Biohaz. 2005. Opinion of the scientific panel on biological hazards on the request from the Commission related to the microbiological risks on washing of table eggs. *EFSA J.* 269.

18. EFSA ECDC. 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. *EFSA J.* 13:1-165.

19. EFSA ECDC. 2016. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. *EFSA J.* 14:4634.

20. Ernst, R. A. 2004. Hatching egg sanitation: The key step in successful storage and production. p. 1-4. Division of Agriculture and Natural Resources, University of California.

21. Farrell, H. P., M. Garvey, M. Cormican, J. G. Laffey, and N. J. Rowan. 2010. Investigation of critical inter-related factors affecting the efficacy of pulsed light for inactivating clinically relevant bacterial pathogens. *J. Appl. Microbiol.* 108:1494-1508.

22. FDA. Date, 2010, Irradiation in the production, processing and handling of food. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=179&showFR=1.

23. Gao, F., L. E. Stewart, S. W. Joseph, and L. E. Carr. 1997. Effectiveness of ultraviolet irradiation in reducing the numbers of *Salmonella* on eggs and egg belt conveyor materials. *Appl. Engin. Agricult*. 13:355-359.

24. Glaser, P., L. Frangeul, C. Buchrieser, C. Rusniok, A. Amend, F. Baquero, P. Berche, H. Bloecker, P. Brandt, T. Chakraborty, A. Charbit, F. Chetouani, E. Couve, A. de Daruvar, P. Dehoux, E. Domann, G. Dominguez-Bernal, E. Duchaud, L. Durant, O. Dussurget, K. D. Entian, H. Fsihi, F. Garcia-Del Portillo, P. Garrido, L. Gautier, W. Goebel, N. Gomez-Lopez, T. Hain, J. Hauf, D. Jackson, L. M. Jones, U. Kaerst, J. Kreft, M. Kuhn, F. Kunst, G. Kurapkat, E. Madueno, A. Maitournam, J. M. Vicente, E. Ng, H. Nedjari, G. Nordsiek, S. Novella, B. de Pablos, J. C. Perez-Diaz, R. Purcell, B. Remmel, M. Rose, T. Schlueter, N. Simoes, A. Tierrez, J. A. Vazquez-Boland, H. Voss,

J. Wehland, and P. Cossart. 2001. Comparative genomics of Listeria species. *Sci.* 294:849-852.

25. Gomez-Lopez, V. M., F. Devlieghere, V. Bonduelle, and J. Debevere. 2005. Factors affecting the inactivation of micro-organisms by intense light pulses. *J. Appl. Microbiol.* 99:460-470.
26. Gomez-Lopez, V. M., P. Ragaert, J. Debevere, and F. Devlieghere. 2007. Pulsed light for food decontamination: a review. *Trends Food Sci. Technol*. 18:464-473.
27. Heir, E., A. L. Holck, M. K. Omer, O. Alvseike, M. Hoy, I. Mage, and L. Axelsson. 2010. Reduction of verotoxigenic *Escherichia coli* by process and recipe optimisation in dry-fermented sausages. *Int. J. Food Microbiol.* 141:195-202.
28. Hierro, E., S. Manzano, J. A. Ordonez, L. de la Hoz, and M. Fernandez. 2009. Inactivation of *Salmonella enterica* serovar Enteritidis on shell eggs by pulsed light technology. *Int. J. Food Microbiol*. 135:125-130.
29. Himathongkham, S., H. Riemann, and R. Ernst. 1999. Efficacy of disinfection of shell eggs externally contaminated with *Salmonella* Enteritidis - Implications for egg testing. *Int. J. Food Microbiol.* 49:161-167.
30. Hutchison, M. L., J. Gittins, A. Walker, N. Sparks, T. J. Humphrey, C. Burton, and A. Moore. 2004. An assessment of the microbiological risks involved with egg washing under commercial conditions. *J. Food Protect*. 67:4-11.
31. Jones, D. R., M. T. Musgrove, and J. K. Northcutt. 2004. Variations in external and internal microbial populations in shell eggs during extended storage. *J. Food Protect*. 67:2657-2660.
32. Keklik, N. M., A. Demirci, P. H. Patterson, and V. M. Puri. 2010. Pulsed UV light inactivation of *Salmonella* Enteritidis on eggshells and its effects on egg quality. *J. Food Protect*. 73:1408-1415.

33. Keklik, N. M., A. Demirci, V. M. Puri, and P. H. Heinemann. 2012. Modeling the inactivation of *Salmonella* Typhimurium, *Listeria monocytogenes*, and *Salmonella* Enteritidis on poultry products exposed to pulsed UV light. *J. Food Protect*. 75:281-288.

34. Krishnamurthy, K., J. C. Tewari, J. Irudayaraj, and A. Demirci. 2010. Microscopic and Spectroscopic Evaluation of Inactivation of Staphylococcus aureus by Pulsed UV Light and Infrared Heating. *Food Bioproc. Technol*. 3:93-104.

35. Kuo, F. L., J. B. Carey, and S. C. Ricke. 1997. UV irradiation of shell eggs: Effect on populations of aerobes, molds, and inoculated *Salmonella* Typhimurium. *J. Food Protect*. 60:639-643.

36. Lasagabaster, A., J. Carlos Arboleya, and I. Martinez de Maranon. 2011. Pulsed light technology for surface decontamination of eggs: Impact on *Salmonella* inactivation and egg quality. *Innov. Food Sci. Emerg. Technol.* 12:124-128.

37. Lasagabaster, A., and I. Martinez de Maranon. 2012. Sensitivity to pulsed light technology of several spoilage and pathogenic bacteria isolated from fish products. *J. Food Protect*. 75:2039-2044.

38. McLeod, A., I. Mage, E. Heir, L. Axelsson, and A. L. Holck. 2016. Effect of relevant environmental stresses on survival of enterohemorrhagic Escherichia coli in dry-fermented sausage. *Int. J. Food Microbiol.* 229:15-23.

39. Nesbakken, T. 1995. *Listeria monocytogenes* in the food chain - a foodborne infection problem common to the Nordic countries. *TemaNord. The Nordic Council of Ministers*. 635:48 p.

40. NMKL NorVal International. 2002. Microbiol. of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp. NMKL NorVal International.

41. Oguma, K., H. Katayama, and S. Ohgaki. 2002. Photoreactivation of *Escherichia coli* after low- or medium-pressure UV disinfection determined by an endonuclease sensitive site assay. *Appl. Environ. Microbiol.* 68:6029-6035.

42. Paskeviciute, E., I. Buchovec, and Z. Luksiene. 2011. High-power pulsed light for decontamination of chicken from food pathogens: A study on antimicrobial efficiency and organoleptic properties. *J. Food Safety*. 31:61-68.

43. Paton, A. W., R. M. Ratcliff, R. M. Doyle, J. SeymourMurray, D. Davos, J. A. Lanser, and J. C. Paton. 1996. Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with Shiga-like toxin-producing *Escherichia coli. J. Clin. Microbiol*. 34:1622.

44. Quek, P. H., and J. Hu. 2008. Influence of photoreactivating light intensity and incubation temperature on photoreactivation of *Escherichia coli* following LP and MP UV disinfection. *J. Appl. Microbiol.*. 105:124-133.

45. R_Core_Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

46. Rodriguez-Romo, L. A., and A. E. Yousef. 2005. Inactivation of *Salmonella enterica* serovar enteritidis on shell eggs by ozone and UV radiation. *J. Food Protect*. 68:711-717.

47. Schimmer, B., K. Nygard, H. M. Eriksen, J. Lassen, B. A. Lindstedt, L. T. Brandal, G. Kapperud, and P. Aavitsland. 2008. Outbreak of haemolytic uraemic syndrome in Norway caused by stx(2)-positive *Escherichia coli* O103 : H25 traced to cured mutton sausages. *BMC Infect. Dis*. 8.

48. Scott, T. A., and C. Swetnam. 1993. Screening sanitizing agents and methods of application for hatching eggs I. Environmental and user friendliness. *J. Appl. Poultry Res*. 2:1-6.

49. Sommers, C. H., J. E. Sites, and M. Musgrove. 2010. Ultraviolet light (254 nm) inactivation of pathogens on foods and stainless steel surfaces *J. Food Safety*. 30:470-479.

50. Takeshita, K., J. Shibato, T. Sameshima, S. Fukunaga, S. Isobe, K. Arihara, and M. Itoh. 2003. Damage of yeast cells induced by pulsed light irradiation. *Int. J. Food Microbiol.* 85:151-158.

51. Turtoi, M., and D. Borda. 2014. Decontamination of egg shells using ultraviolet light treatment. *Worlds Poultry Sci. J.* 70:265-277.

52. Wekhof, A., F.-J. Tropeter, and O. Franken. 2001. Pulsed UV disintegration (PUVD): a new sterilisation mechanism for packaging and broad medical-hospital applications. p. 1-15. The first international conference on ultraviolet technologies, Washington DC, USA.

53. Woodling, S. E., and C. I. Moraru. 2005. Influence of surface topography on the effectiveness of pulsed light treatment for the inactivation of *Listeria innocua* on stainless-steel surfaces. *J. Food Sci.* 70:M345-M351.

54. Xu, L., C. Tian, X. Lu, L. Ling, J. Lv, M. Wu, and G. Zhu. 2015. Photoreactivation of *Escherichia coli* is impaired at high growth temperatures. *J. Photochem. Photobiol. B-Biol*. 147:37-46.

55. Zhang, W., J. X. Zheng, and G. Y. Xu. 2011. Toward better control of *Salmonella* contamination by taking advantage of the egg's self-defense system: A review. *J. Food Sci.* 76:R76-R81.

FIGURE LEGENDS

FIGURE 1. Reduction of *Salmonella* on clean eggs by UV-C (white bars) and pulsed UV (gray bars) light treatments. (A) *Salmonella* from overnight cultures were mixed and applied directly onto 8 cm^2 of the egg surface. (B) *Salmonella* from overnight cultures were washed twice in distilled water before being applied onto 8 cm^2 of the egg surface. (C) Washed *Salmonella* cells were applied onto 3 mm^2 of the egg surface. The UV fluences are indicated and correspond to 10 mW/cm^2 for 5, 10, 30, 60 and 300 s for UV-C light. Samples with upper and lower case letters were analyzed separately by ANOVA, and samples containing the same letter were not considered different. Letters A to D and a-d were used to compare treatments of unwashed and washed cells, while J to M and j to m were used to compare treatments of washed cells on large and small areas.

FIGURE 2. Reduction of *Salmonella* on eggs with chicken feces by UV-C (white bars) and pulsed UV (gray bars) light treatments. *Salmonella* was mixed with chicken feces and applied onto 4 cm^2 of the surface of eggs in layers of different thicknesses, (A) 0.025 mm, (B) 0.05 mm, (C) 0.1 mm, as shown on eggs (D) from left to right, respectively. The UV fluences are indicated and correspond to 10 mW/cm^2 for 5, 10 and 60 s for UV-C light. Samples with upper and lower case letters were analyzed separately by ANOVA, and samples containing the same letter were not considered different.

FIGURE 3. Reduction of (A) *L. monocytogenes* and (B) EHEC on clean eggs by UV-C (white bars) and pulsed UV (gray bars) light treatments. Cells from overnight cultures were mixed and applied onto 8 cm^2 of the egg surface. The UV light fluences are indicated and correspond

to 10 mW/cm^2 for 5, 10, 30, 60 and 300 s for UV-C light. Samples with upper and lower case letters were analyzed separately by ANOVA, and samples containing the same letter were not considered different. ANOVA was performed with *L. monocytogenes*, EHEC and *Salmonella* Enteritidis (results from Fig. 1A) as levels in a factor of a joint analysis.

FIGURE 4. Scanning electron microscopy of eggshell surfaces after UV light treatments. (A) UV-C light treatment at fluence 0.6 J/cm^2 (10 mW/cm^2, 60 s), (B) Pulsed UV light treatment, at fluence 10.8 J/cm^2, (C) Untreated control.

FIGURE 5. Sensory analysis of (A) intact eggs and (B) egg content. Eggs were exposed to UV-C light at fluences 0.1 J/cm^2 (CUV-10, 10 mW/cm^2, 10 s) and 0.6 J/cm^2 (CUV-60, 10 mW/cm^2, 60 s) and to pulsed UV light at fluences 1.25 J/cm^2 (P-UV-L) and 10.8 J/cm^2 (P-UV-3xH). The intensity of different odours of intact eggs were registered. The eggs were then opened and the contents of the same eggs were evaluated. 1 = low intensity, and 9 = high intensity. The letters above the columns indicate grouping according to ANOVA and Tukey multiple comparison test. Samples with the same letter are considered being equal for the specific property. Underlined A indicates that all intensities belong to the same group for that specific property.

TABLE 1. Strains used in this study.

Species	Strain/Serotype	Source	MF number Rif^{R},[1]
S. Enteritidis	1049-1-99	Norwegian Veterinary Institute, Norway	3817
	61-358-1	DTU Vet, National Veterinary Institute, Denmark	3818
		ATCC13076[2]	3824
L. monocytogenes	2230/92	Meat product, caused food poisoning in Norway 1992 *(39)*	3508
	167	Knife in meat factory *(10)*	3509
	187	Meat product *(10)*	3510
	EGD-e	*(24)*	3571
EHEC	O103:H25	Rif mutant of MF2522. From dry fermented sausage. Linked to outbreak in Norway 2006 *(47)*	3572
	O157:H7	ATCC43895[2]	3574
	O111:H^{-}	Semi-dry fermented sausage, outbreak in Australia 1995 *(43)*	3576
	O145	Rif mutant of MF2493, Human clinical strain, sporadic *(38)*	5554

[1]Reference number of strains after mutating to Rif^{R}.

[2]American Type culture Collection, Manassas, VA, USA

TABLE 2. Testing for increased permeability after UV-C and pulsed UV light treatments of eggs.

Treatment	*Salmonella* positive/total[a]
Continuous UV-C, 3.0 J/cm^2	0/13
Pulsed UV, 10.8 J/cm^2	0/12
No UV, control[b]	0/13
Contaminated control[c]	2/2

[a]Qualitative determination of *Salmonella* according to NMKL ISO 6579:2002.

[b]Eggs contaminated with *Salmonella* on outside

[c]Eggs deliberately contaminated with *Salmonella* in the egg white.